



## Loving-Kindness Meditation practice associated with longer telomeres in women

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### ABSTRACT

Relatively short telomere length may serve as a marker of accelerated aging, and shorter telomeres have been linked to chronic stress. Specific lifestyle behaviors that can mitigate the effects of stress might be associated with longer telomere lengths. Previous research suggests a link between behaviors that focus on the well-being of others, such as volunteering and caregiving, and overall health and longevity. We examined relative telomere length in a group of individuals experienced in Loving-Kindness Meditation (LKM), a practice derived from the Buddhist tradition which utilizes a focus on unselfish kindness and warmth towards all people, and control participants who had done no meditation. Blood was collected by venipuncture, and Genomic DNA was extracted from peripheral blood leukocytes. Quantitative real time PCR was used to measure relative telomere length (RTL) (Cawthon, 2002) in fifteen LKM practitioners and 22 control participants. There were no significant differences in age, gender, race, education, or exposure to trauma, but the control group had a higher mean body mass index (BMI) and lower rates of past depression. The LKM practitioners had longer RTL than controls at the trend level ( $p = .083$ ); among women, the LKM practitioners had significantly longer RTL than controls, ( $p = .007$ ), which remained significant even after controlling for BMI and past depression. Although limited by small sample size, these results offer the intriguing possibility that LKM practice, especially in women, might alter RTL, a biomarker associated with longevity.

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### 1. Introduction

Telomeres are nucleoprotein structures located at the ends of chromosomes which shorten with repetitive cell division and replication. Generally, telomeres shorten with age, and this shortening may be accelerated in the presence of cellular oxidative damage or chronic psychological stress (Damjanovic et al., 2007; Epel et al., 2004; Kotschal et al., 2007). For example, caretakers of family members with Alzheimer's dementia had shorter telomeres than age and gender matched controls, and patients with chronic mood disorders had shorter telomeres than age and gender matched controls (Damjanovic et al., 2007; Simon et al., 2006).

Several studies have now demonstrated that higher levels of psychological and life stress are associated with shorter telomeres, such as a history of intrauterine stress (Entringer et al., 2011), childhood adversity (Kananen et al., 2010; Tyrka et al., 2010)

(although not always (Glass et al., 2010)), high phobic anxiety (Okereke et al., 2012), severe job stress (Ahola et al., 2012), concurrent stress and chronic pain (Sibille et al., 2012), and poor sleep (Prather et al., 2011). Notably, shorter telomeres have been associated with earlier mortality (Cawthon et al., 2003). Therefore, telomere length may reflect the association of psychological well-being on health and longevity.

It is not clear whether lifestyle factors can increase telomere length or slow the shortening of telomeres, but there are studies that show associations between health behaviors or lifestyle choices and telomere length or telomerase, the enzyme that helps repair telomeres by adding DNA hexameric repeats to restore telomere length. For example, physical exercise has been shown to be associated with longer telomeres and higher telomerase activity, and was found to moderate the effect of stress on telomere length (Ludlow et al., 2008; Puterman et al., 2010; Werner et al., 2009). A wide variety of healthy behaviors and health related factors have been associated with longer telomeres (body mass index below 25 kg/m<sup>2</sup>, non-smoking, healthy diet, and moderate to vigorous exercise) (Sun et al., 2012). Lastly, being married has been associated with longer telomere length at trend-level significance ( $p = 0.067$ , (Yen and Lung, 2012)).

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There is evidence that meditation training could also affect telomerase. In a longitudinal study of overweight or obese women, mindfulness meditation was associated with a 39% increase in telomerase activity over 4 months, which was a 18% greater increase in telomerase activity compared to a control group, although this difference did not reach significance (Daubenmier et al., 2012). In another longitudinal study, older adults who took care of persons with dementia were taught a type of yogic meditation (Kirtan Kriya) or given music to listen to. The yogic meditation group had a greater rise in telomerase activity compared to the group who listened to music (Lavretsky et al., 2013). A study of intensive daily meditation practice showed higher cross-sectional telomerase activity in a group of individuals at the end of an intensive three-month full-time meditation retreat, compared to a waitlist control group (Jacobs et al., 2011). Concurrent psychometric measures were associated with greater telomerase activity: increases in Perceived Control, decreased Neuroticism, and increased Purpose in Life.

The above findings suggest that meditation training could potentially have a protective effect on telomeres. A strong theoretical basis has been forwarded that suggests that mindfulness meditation training could have beneficial effects on telomeres by reducing cognitive stress and increasing positive states of mind (Epel et al., 2009). This hypothesis is supported by data from multiple trials that suggest that meditation practice reduces psychological stress (Kvillemo and Branstrom, 2011; Manocha et al., 2011; Smith et al., 2008).

If meditation practice protects telomeres against accelerated aging due to stress, we would expect to see longer telomeres in long-term experienced meditators. Data from other methodologies suggest that meditation could have a protective effect against aging, including a study showing a lack of age-related atrophy of brain grey matter (Lazar et al., 2005).

Loving-Kindness or *metta* meditation, (*metta* from the Pali language of the Buddhist scripture), is a type of meditation practice that focuses on developing a positive intention, unselfish kindness and warmth towards all people (Salzberg, 1995). Preliminary work on Loving-Kindness Meditation (LKM) has demonstrated positive effects of this practice. For example, employees who signed up for a workplace wellness program were randomized to learn LKM or were placed on a waitlist. After 7 weeks, individuals in the LKM program had more positive emotions, a sense of purpose in life, social support, and decreased illness symptoms such as headaches, congestion or weakness (Fredrickson et al., 2008). In a pilot study of patients with chronic low back pain randomized to LKM or standard care, LKM was associated with greater decreases in pain, anger, and psychological distress than the control group (Carson et al., 2005). The above data taken together suggest that LKM, a practice that promotes positive feelings towards others, can improve overall health. Because shorter telomeres are associated with chronic psychological stress, and LKM appears to decrease stress, we examined telomere length in a population of experienced LKM meditators, and hypothesized that they would have longer telomeres than age, gender, and education-matched controls. In addition, since evidence from the literature suggests that telomere length is longer in women (Bekaert et al., 2007; Nawrot et al., 2004), we decided to also analyze genders separately.

## 2. Methods

### 2.1. Participants

Individuals over the age of 18 with extensive training in LKM practice were recruited with print advertisements and flyers asking for meditators experienced in Metta (LKM) from Vipassana

meditation communities and retreat centers in New England. To participate, individuals had to have 4 years or more of regular, nearly daily LKM practice, and must have attended at least one overnight meditation retreat (not necessarily LKM) of 3 days or more duration. Control participants were also age 18 or above, but were required not to have experience with any meditation or yoga practices (no more than 4 classes over the lifetime).

Exclusion criteria included the current diagnosis of any of the following mental disorders as defined by the DSM-IV and assessed by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998): schizophrenia or other psychosis, mental retardation, post-traumatic stress disorder, alcohol or substance abuse or dependence, major depression, panic disorder, or generalized anxiety disorder. Participants were also excluded if they had a serious current medical illness, if they took hormonal medication such as oral contraceptives or hormone replacement therapy, had endocrine diseases such as Addison's or Cushing's disease, had an acute inflammatory disease, had a diagnosis of cancer in the last 5 years, or were pregnant or lactating.

We recruited 75% of the LKM group first, and then recruited controls that resembled the LKM group, targeting the mean age ( $\pm 5$  years), gender proportions, education level, and levels of depression history that was observed in the LKM group. Several individuals in the LKM group had a history of past major depressive episodes, and experienced meditators were also found to be highly educated; many had a graduate degree. Therefore, in order to prevent confounding, we over-enrolled control participants that had a past history of depression and a graduate level of education.

### 2.2. Procedure

#### 2.2.1. Blood collection

Subjects arrived at the laboratory between 1 pm and 3 pm. Blood was obtained by venipuncture from an antecubital vein and collected into tubes containing Ficoll gel for separation of leukocytes by centrifugation. Leukocytes were rinsed in phosphate buffer solution and the remaining cell pellet stored at  $-80$  degrees Celsius.

### 2.3. Relative leukocyte telomere length measurement

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp 96-spin DNA blood protocol (QIAGEN, Valencia, CA). Quantitation of genomic DNA with PicoGreen was performed using a Molecular Devices 96-well spectrophotometer. To assess relative leukocyte telomere length, quantitative real-time polymerase chain reaction (McGrath et al., 2007) was performed on the 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Drying down 5 ng of the genomic DNA and resuspending it in 10  $\mu$ L of either telomere or single-gene (36B4) PCR reaction mix standardized the samples. The telomere PCR reaction mix consists of 1  $\times$  QuantiTect SYBR Green PCR Master Mix, 2.5 mM of DTT, 270 nM Tel-1b primer, and 900 nM of Tel-2b primer. Assays proceeded for 1 cycle at 95  $^{\circ}$ C for 10 min, followed by 30 cycles at 95  $^{\circ}$ C for 15 s and 54  $^{\circ}$ C for 2 min. The 36B4 PCR reaction mix consists of 1  $\times$  QuantiTect SYBR Green PCR Master Mix, 300 nM 36B4u primer and 500 nM 36B4d primer. The 36B4 thermal cycling proceeded with 1 cycle at 95  $^{\circ}$ C for 10 min, followed by 30 cycles at 95  $^{\circ}$ C for 15 s and 58  $^{\circ}$ C for 1 min and 10 s. Samples were assayed in triplicate. Blinded quality control samples were interspersed throughout the plate to assess variability. As described previously, the average relative leukocyte telomere length (LTL) is reported as the exponentiated sample ratio of telomere copy number to a 36B4 copy number (T/S) corrected for a reference sample (Cawthon, 2002). Coefficients of variation (CVs) for the telomere and single gene assay were less than 2%, and CVs for the exponentiated T/S

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